GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA

GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 08 | Issue: 03

On 30th September 2022

http://www.research.lk

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GARI Publisher | Health | Volume: 08 | Issue: 03

Article ID: IN/GARI/ICAS/2022/112 | Pages: 65-74 (10)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 13.06.2022 | Publish: 30.09.2022

SITE-SPECIFIC DOCKING AGAINST HEPATITIS B PROTEIN USING AUTODOCK 4.2.6.

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ABSTRACT

The hepatitis B virus (HBV) is one of the major causes of liver diseases. There are several interventions to manage the condition, but no effective cure. In silico screening is an excellent tool to discover novel drug candidates to treat HBV. In this study site-specific, protein-ligand docking was performed using Autodock 4.2.6. to determine phytochemicals that can target the hepatitis B core (HBc) protein (PDB ID:5WRE). Ten phytochemicals (sanggenol O, helioxanthin, curcumin, nirtetralin, neolancerin, dehydrozingerone, LPRP-ET-97543, myricetin-3-o-rhamnoside, quercetin-3-o-rhamnoside and kaempferol) were selected and a clinical trial drug (NVR-3-778) was used as a standard. When docking a grid box was created, where its xyz-dimensions were 60 each, grid spacing was 0.5 and genetic algorithm runs was set to 50. Re-docking was conducted to validate the procedure. The ligands were evaluated based on their binding energies (BE) and inhibitory constants (Ki). The best-docked poses and amino acid interactions were visualized using BIOVIA Discovery Studio. Moreover, SwissADME was used to analyze the pharmacokinetic properties. The best-docked phytochemicals were sanggenol O, helioxanthin and LPRP-ET-97543 as they had the lowest BE's of -9.73kcal/mol, -8.67kcal/mol and -8.18kcal/mol respectively while their Ki's were 0.07416μM, 0.43846μM and 1.01μM, respectively. The re-docked BE was -10.19kcal/mol. The common amino acid interactions observed were TYR118, VAL124, LEU140 and PHE110. Furthermore, these phytochemicals conformed to Lipinski's criteria and showed high gastrointestinal absorption indicating that they have a good bioavailability. In conclusion these three phytochemicals displayed stable BE's, potent Ki's and conformed to Lipinski's criteria suggesting that they are suitable ligands for therapeutic use.

Key words: Hepatitis B core (HBc) protein, Site-specific docking, Binding energy (BE), Inhibitory constant (Ki), Lipinski's criteria

INTRODUCTION

The hepatitis B virus (HBV) is a DNA virus, belonging to the Hepadnaviridae family, which causes liver damage leading to cirrhosis and hepatocellular carcinoma (HCC). Approximately 269 million people are infected with hepatitis B worldwide (Sonderup and Spearman, 2022). Regions of Africa, South East Asia and South America have an intermediate to high prevalence, however, compared to most Asian countries, Sri Lanka was revealed to be the least affected by HBV with a prevalence of less than 2% (Noordeen, Pitchai and Rafeek, 2015; Stasi, Silvestri and Voller, 2017). Interfenons and nucleoside analogues are the current treatment interventions for HBV. They help manage the condition but do not offer are permanent cure. Interfenons have an immunomodulatory effect, hence they indirectly inhibit viral replication, while nucleoside analogues inhibit specific

stages of the viral life cycle such as disrupting the DNA polymerase activity, thereby preventing the synthesis of viral proteins (Wong, Wong and Chan, 2014).

It is imperative to find out new treatments for HBV as it is a major contributory factor to HCC, which is the second most common cause of cancer-related deaths (Sayiner, Golabi and Younossi, 2019). Furthermore, prolonged use of antiviral drugs leads to resistance, for instance, genotypic resistance up to 70% was detected after administration of lamivudine for five years (Khungar and Han, 2010). The HBV is composed of a lipid bi-layer, a viral nucleocapsid protein that encloses a 3.2kb long double-stranded DNA and the polymerase enzyme (Busch and Thimme, 2014). The DNA contains four overlapping open read frames (ORFs) that code for seven proteins, namely, the three envelope proteins large (L), medium (M) and small (S), the hepatitis B X protein, the polymerase protein, pre-core and core proteins (Kashyap, Tiwari and Prakash, 2018). By targeting these proteins, a solution can be found for this disease. The hepatitis B core (HBc) protein plays a significant role in several stages of viral development. The HBc protein is required for the construction of the nucleocapsid which is necessary to protect the viral genome. It is also involved in the transportation of the viral particle to the nucleus as well as mediating the release of the relaxed circular DNA (rcDNA) for the synthesis of the covalently closed circular DNA (cccDNA) for viral replication to proceed (Viswanathan et al., 2020).

The development of a class of drugs known as capsid assembly modulators (CAM) is underway and NVR 3-778 is one of the first of these drugs to produce promising results in preclinical trials. NVR 3-778 had successfully suppressed viral replication in primary human hepatocytes by inhibiting the encapsidation of the pregenomic RNA (Lam et al., 2018). A study showed that the combination of NVR 3-778 with pegylated interfenon had a synergistic effect as it greatly reduced the viral load (Dawood et al., 2017). Hence in silico screening is an excellent, cost-effective method for the discovery of novel lead compounds to target the HBc protein. Protein-ligand docking is a popular technique to determine binding stability and protein-ligand interactions. Autodock 4.2.6. is a freely accessible and commonly used docking software in the field of drug design and development. This study aimed to evaluate the binding efficiency of ten phytochemicals and to narrow down the top-hit compounds using Autodock 4.2.6.

MATERIALS

The hardware consisted of a Dell laptop with an Intel(R) Core(TM) i5-3337U CPU @ 1.80GHz, 64-bit processor, an 8GB RAM and Windows 10 operating system. The software used were Autodock 4.2.6., Open Babel GUI 2.4.1 and BIOVIA Discovery Studio (DS) Visualizer 21.1.1.0.0. Additionally, MGL Tools 1.5.6 and Python 3.10.0 were installed as supporting software for Autodock 4.2.6. to run. The web tools consisted of the NCBI Pubchem database, Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) and SwissADME. The 3D structure of the HBc core protein (PDB ID: 5WRE) consisting of a resolution of 1.95Å was obtained from the PDB website, while the ligands (Table 1) were acquired from NCBI Pubchem. These ligands were selected as they have shown antiviral properties in previously conducted wet-lab research as well as docking studies.

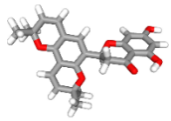
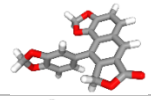
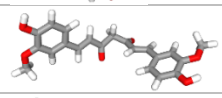
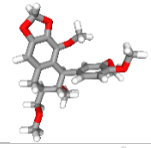
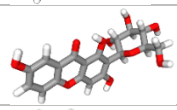
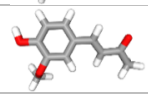
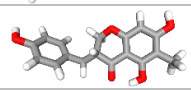
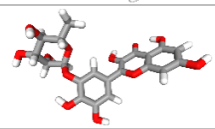
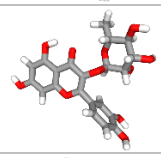
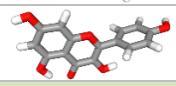
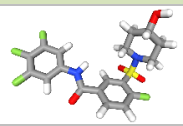
Ligands	Pubchem CID	Structure	References
Phytochemicals			
Sanggenol O	42608050		Ivonie, Yanuar and Firdayani, 2018
Helioxanthin	177023		Wu, 2016
Curcumin	969516		Kim *et al*., 2009
Nirtetralin	182644		Wei *et al.*, 2012
Neolancerin	92029590		Liu *et al*., 2020
Dehydrozingerone	5354238		He *et al.*, 2016
LPRP-Et-97543	71585044		Huang *et al*., 2014
Myricetin-3-O-rhamnoside	56843093		Parvez *et al*., 2020
Quercetin-3-O-rhamnoside	15939939		Parvez *et al*., 2021
Kaempferol	5280863		Parvez *et al*., 2021
Clinical trial drug			
NVR 3-778	89663273		Lam *et al*., 2018

Table 1: List of ligands and their Pubchem CID's

METHODOLOGY

Protein preparation

The receptor was downloaded in the .pdb format. Using Autodock 4.2.6., the water molecules and chains A, D, E and F were deleted. The heteroatoms isopropyl alcohol (IPA), glycerol (GOL), (2S)-1-[[(4R)-4-(2-chloranyl-4-fluoranyl-phenyl)-5-methoxycarbonyl-2-(1,3-thiazol-2-yl)-1,4-dihydropyrimidin-6-yl]methyl]-4,4 bis(fluoranyl)pyrrolidine-2-carboxylic acid (7TL), chloride ions (Cl) were removed from chains B and C. The missing atoms were detected and repaired, followed by the addition of polar hydrogen bonds and Kollman charges. This file was saved as a .pdbqt file.

Ligand preparation

The ligands were acquired in .sdf format and converted to a .pdb file using Open Babel GUI. Upon opening the file in Autodock 4.2.6. Gasteiger charges were automatically added and the number of rotatable bonds were calculated. Torsions were set and the ligands were saved in a .pdbqt file.

Protein-ligand docking

A grid box was created to enclose a specific region of the B and C chains. The grid dimensions were adjusted as follows; x=60, y=60 and z=60, while the grid center values were x=-26.431, y=-9.648 and z=-6.647 and its spacing was 0.5. The docking search parameter chosen was the Lamarckian genetic algorithm where the genetic algorithm runs was set to 50 with a population size of 300. Subsequently, Autogrid was executed, which produced grid maps for individual atoms of the ligand to be docked. This was followed by the execution of Autodock, which uses the grid maps to deduce the protein-ligand interactions (Goodsell et al., 1996). A docking log (DLG) file was produced, containing the binding energies (BE) and inhibitory constant (Ki) values for all the poses generated per ligand.

Re-docking

The co-crystal 7TL was extracted using BIOVIA DS by deleting all the chains, water molecules and heteroatoms except 7TL. It was then saved in a .pdb format. The co-crystal was subjected to site-specific docking using the same procedure conducted for the test ligands.

Analysis of pharmacokinetic properties

The canonical SMILEs of the phytochemicals were acquired from the Pubchem database (Table 2). These were entered into SwissADME, which predicted the absorption, distribution, metabolism and excretion (AMDE) properties. Lipinski's rule of five was applied to predict their drug likeness.

Ligands	Canonical SMILEs
Sanggenol O	CC1(C=CC2=CC(=C3C(=C2O1)C=CC(O3)(C)C)C4CC(=O)C5=C(C=C(C=C5O4)O)O)C
Helioxanthin	C1C2=C(C=C3C=CC4=C(C3=C2C5=CC6=C(C=C5)OCO6)OCO4)C(=O)O1
Curcumin	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O
Nirtetralin	COCC1CC2=CC3=C(C(=C2C(C1COC)C4=CC(=C(C=C4)OC)OC)OC)OCO3
Neolancerin	C1=CC2=C(C=C1O)C(=O)C3=C(O2)C=C(C(=C3O)C4C(C(C(C(O4)CO)O)O)O)O
Dehydrozingerone	CC(=O)C=CC1=CC(=C(C=C1)O)OC
LPRP-Et-97543	CC1=C(C2=C(C=C1O)OCC(C2=O)CC3=CC=C(C=C3)O)O
Myricetin-3-O-rhamnoside	CC1C(C(C(C(O1)OC2=CC(=CC(=C2O)O)C3=C(C(=O)C4=C(C=C(C=C4O3)O)O)O)O)O)O
Quercetin-3-O-rhamnoside	CC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC(=C(C=C4)O)O)O)O)O
Kaempferol	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O

Table 2: Canonical SMILES

RESULTS

Docking results

The BE of the re-docked 7TL co-crystal was -10.19kcal/mol. The best ligands were deduced based on their BE values and their Ki. Table 1 displays the BE's and Ki's of the docked compounds. Sanggenol O, helioxanthin and LPRP-Et-97543 were the best-docked ligands as they have the lowest BE's of, -9.73kcal/mol, -8.67kcal/mol and -8.18kcal/mol, respectively. Additionally, they had the lowest Ki's, with sanggenol O being the most potent as its Ki was even lower than the clinical trial drug.

Ligands	BE (kcal/mol)	Ki (μM)
Phytochemicals		
Sanggenol O	-9.73	0.07416
Helioxanthin	-8.67	0.43846
Nirtetralin	-7.48	3.3
Curcumin	-7.43	3.59
Neolancerin	-6.73	11.64
Dehydrozingerone	-6.04	37.63
LPRP-Et-97543	-8.18	1.01
Myricetin-3-O-rhamnoside	-6.28	24.76
Quercetin-3-O-rhamnoside	-7.58	2.79
Kaempferol	-6.66	13.05
Clinical trial drug		
NVR-3-778	-11.1	0.10692

Table 3: Docking results

Visualization

The 3D ribbon structures of the best-docked poses were generated using BIOVIA DS, which is depicted in Figure 1. Chain B is represented in blue, chain C is shown in green and the ligands are displayed in orange.

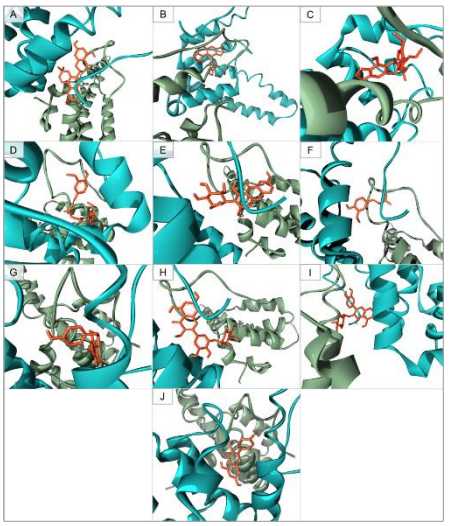


Figure 1: 3-dimensional ribbon structures of sanggenol O (A), helioxanthine (B), nirtetralin (C), curcumin (D) neolancerin (E), dehydrozingerone (F), LPRP-Et-97543 (G), myricetin-3-O-rhamnoside (H), quercetin-3-O-rhamnoside (I) and kaempferol (J)

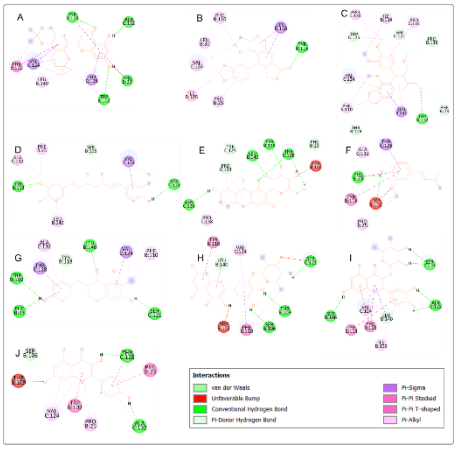


Figure 2: Amino acid interactions of sanggenol O (A), helioxanthine (B), nirtetralin (C), curcumin (D) and neolancerin (E), dehydrozingerone (F), LPRP-Et-97543 (G), myricetin-3-O-

rhamnoside (H), quercetin-3-O-rhamnoside (I) and kaempferol (J)

The common amino acid interactions of sanggenol O, helioxanthine and LPRP-Et-97543 observed were TYR118, VAL124, LEU140 and PHE110 (Figure 2). Similar results were obtained from previous research where the amino acid interactions were TYR118, VAL124, THR128, LEU140, PHE110, ALA132, TRP102, PHE23, THR33, ILE105, LEU30, PHE122 and SER121 (Ivonie, Yanuar, Firdayani, 2018). Thus suggesting that these amino acids are necessary for ligands to successfully dock

.

ADME property analysis

Ligands	MW(g/mol)	Rot-bonds	HBA	HBD	TPSA(Å²)	Lipophilicity (MLOGP)	GI absorption	F score
Sanggenol O	420.45	1	6	2	85.22	2.28	High	0.55
Helioxanthin	348.31	1	6	0	63.22	2.79	High	0.55
Nirtetralin	430.49	8	7	0	64.61	1.91	High	0.55
Curcumin	368.38	8	6	2	93.06	1.47	High	0.55
Neolancerin	406.34	2	10	7	181.05	-2.16	Low	0.55
Dehydrozingerone	192.21	3	3	1	46.53	1.33	High	0.55
LPRP-Et-97543	300.31	2	5	3	86.99	1.20	High	0.55
Myricetin-3-O-rhamnoside	464.38	3	12	8	210.51	-1.84	Low	0.17
Quercetin-3-O-rhamnoside	448.38	3	11	7	190.28	-1.84	Low	0.17
Kaempferol	286.24	1	6	4	111.13	-0.03	High	0.55

Table 4: SwissADME parameters

Abbreviations: MW: Molecular weight, Rot-bonds: number of rotatable bonds, HBA: hydrogen bond acceptors, HBD Hydrogen bond donors, TPSA: topological polar surface area, GI: gastrointestinal, F: bioavailability

The drug likeness was assessed based on Lipinski's rule of five. For a compound to have a good drug-likeness it must fulfill the following criteria; MW <500 g/mol, MlogP<4.15, H-bond donors < 5, H-bond acceptors<10 (Lipinski et al., 1997; Brito, 2011). A compound that violates two or more of Lipinski's rules is considered to have low oral activity (Tian et al., 2015). Myricetin-3-O-rhamnoside and quercetin-3-O-rhamnoside deviated from Lipinski's rule because both displayed a high number of HBA and HBD (Table 2).

The TPSA and number of rot-bonds were analyzed as they too influence bioavailability. For a compound to have a good bioavailability they must have a TPSA less than 140Å² and number of rot-bonds below 10 (Verber et al., 2002). All the phytochemicals conformed with this except for neolancerin, myricetin-3-O-rhamnoside and quercetin-3-O-rhamnoside as they had a TPSA exceeding 140Å².

DISCUSSION

A high resolution protein structure is required to limit docking errors. Resolutions ranging between 0.5-1 Å are classed as ultra-high, while those within 1.5-2Å are considered as high, and resolutions exceeding 2Å are low (Dubach and Guskov, 2020). Since the 5WRE protein structure for this study had a high resolution of 1.95Å, its 3D structure has

been accurately predicted leading to fewer docking errors.

During the protein preparation, water molecules and heteroatoms were deleted as they hinder the ligand binding. The chains A, D, E and F were removed as the B and C chains were identified to have a good binding pocket for ligands (Zhou et al., 2017; Ivonie et al., 2018). Moreover, missing atoms were detected for and repaired as PDB files can have missing atoms. Kollman and Gasteiger charges were added to the protein and ligands respectively to calculate their atomic charges. The re-docking was conducted to validate the procedure, which confirmed the accuracy of pose prediction as 7TL docked to the HBc protein with stable BE of -10.19kcal/mol.

The best-docked phytochemicals were chosen based on low BE because the lower the BE the more stable the protein-ligand binding. Table 5 compares the results from the current and a previous study for sanggenol O, which shows that consistent results were obtained.

Ligand	BE from current study	BE from previous study	Reference
Sanggenol O	-9.73	-10.58	Ivonie *et al*., 2018

Table 5: Comparison of BE's from current and previous docking studies

The different types of interactions such as hydrogen bonds and hydrophobic bonds like pi-stacking and pi-sigma were observed because, hydrogen bonds are necessary to promote the binding specificity of ligands, while hydrophobic bonds contribute to ligand binding efficiency (Nittinger et al., 2017; Freitas and Schapira, 2017). Sanggenol O and LPRP-Et-97543 exhibited four hydrogen bonds, hence they have a greater specificity compared to helioxanthin, which only contains one hydrogen bond. Ligands with an unfavorable bump displayed the weakest BE's within the -6kcal/mol range because the presence of unfavorable bumps is an indication of repulsive forces, which reduces the stability of the protein-ligand complex.

Compliance with Lipinski's criteria suggests that the phytochemicals have a good bioavailability and are orally active (Chen et al., 2020). Moreover, a TPSA above 140Å² and rotatable bonds above 10 are known to greatly reduce absorption (Verber et al., 2002). The phytochemicals that conformed with Lipinksi's criteria and had TPSA and number of rot-bonds within the cut-off values had a high bioavailability score of 0.55, while those that deviated had a weak score of 0.17. Protein-ligand docking proved to be a quick and inexpensive technique to predict potential small molecules against the HBc protein. Despite these positive results protein-ligand docking comes with its challenges, for instance, most docking techniques treat the protein as a rigid structure, when in reality protein structures can be flexible in specific environmental conditions (Klebe, 2006). Therefore, wet-lab procedures are required to better evaluate the binding of these phytochemicals.

CONCLUSION

In conclusion, the best-docked phytochemicals were sanggenol O, helioxanthin and LPRP-Et-97543 as they had the lowest BE and Ki values. Furthermore, they showed stable amino acid interactions, with sanggenol O and LPRP-Et-97543 displaying a high specificity as they have four hydrogen contacts. They conformed to Lipinski's criteria, implying that they are orally active. They displayed a high bioavailability score of 0.55 as well as a

high GI absorption. Thus suggesting that they have the potential to heal HBV.

In vitro and in vivo tests are necessary to further assess their pharmacokinetic properties, efficacy, potency and safety. The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) colorimetric assay, a common toxicity assay, can be conducted to evaluate cell viability (Kamiloglu et al., 2020). Moreover, a pharmacokinetic profile of these phytochemicals can be deduced using Hepatitis B infected mice models (Amblard et al., 2020).

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